Exposure of Juvenile Chinook and Chum Salmon to Chemical Contaminants in the Hylebos Waterway of Commencement Bay

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Introduction

Estuaries are critical habitats for juveniles of several Pacific salmon species during their transition from fresh water to the ocean (Healy 1982). Estuarine habitats provide refuge from predators, a rich food supply to support rapid growth, and are where juvenile salmon make the adjustment from freshwater to marine conditions (Dorcey et al. 1978, Simenstad et al. 1982). Urban estuaries, however, receive inputs of toxic anthropogenic substances from a variety of sources, and many of these chemicals can accumulate in sediments (Dexter et al. 1985) and thus can be retained in the estuary. There is concern that, because juvenile salmon are undergoing numerous physiological adaptations during their residence in estuarine environments, any additional stresses, such as exposure to toxic chemicals, may be injurious.

The Hylebos Waterway located in Commencement Bay of Puget Sound is a contaminated estuary. Juvenile chinook and chum salmon inhabit this waterway in the late spring and early summer. During 1989 and 1990, juvenile chinook salmon were collected from three Commencement Bay waterways other than the Hylebos Waterway (Stein et al. 1995; Varanasi et al. 1993), and found to be substantially exposed to a variety of contaminants, including polycyclic aromatic hydrocarbons (PAHs) and chlorinated hydrocarbons (CHs). In addition, other studies from the Duwamish Waterway (Stein et al. 1995; Varanasi et al. 1993; McCain et al. 1990) showed levels of chemical contaminant exposure in juvenile chinook salmon similar to that found in the 1989 and 1990 studies of Commencement Bay. Juvenile chinook salmon from the Duwamish Waterway exhibit a variety of biological effects associated with their residence in this contaminated estuary, including reduced immunocompetence, increased mortality after disease challenge, and reduced growth (Varanasi et al. 1993; Arkoosh et al. 1991; Arkoosh et al. in press). Juvenile salmon from the Duwamish Waterway also have increased induction of hepatic cytochrome P4501A (CYP1A) and higher levels of DNA damage compared to juveniles from nonurban estuaries (Stein et al. 1995, Varanasi et al. 1993). Additionally, a recent laboratory investigation demonstrated that immunocompetence of juvenile chinook salmon can be impaired by exposure to CHs and PAHs (Arkoosh et al. 1994).

Prior to the current investigation, however, nothing was known about the potential for chum salmon to be exposed to chemical contaminants during residence in contaminated estuaries, or what the effects of increased exposure might have been on this species. Most importantly, there was no information on exposure of salmonids during residence in the Hylebos Waterway. Accordingly, the aim of the present investigation was to determine to what degree juvenile chum and chinook salmon from the Hylebos Waterway might be exposed to organic contaminants, and to compare the levels of exposure observed to previous studies where such exposures have been linked to biological dysfunction.

Methods

Sample Collection

Juvenile chum and chinook salmon were collected from the Hylebos Waterway, and from reference hatcheries and estuaries considered to be relatively unimpacted by contaminants (Varanasi et al. 1993). Hylebos Waterway juvenile salmon were collected primarily within sight of the 11th Street Bridge, and

fish were sampled weekly for six weeks in May and June, 1994. Fish were sampled once from each reference hatchery and estuary. Reference sites for chum salmon were the Puyallup Tribal Hatchery and the Skokomish Estuary. Reference sites for the chinook salmon were the Puyallup State Hatchery, the Nisqually Estuary, and the Nisqually Hatchery (however, no stomach contents were available for analyses from the Nisqually Hatchery). Beach seines were used to collect the juvenile salmon from the Hylebos Waterway and reference estuaries (PTI 1990; Varanasi et al. 1993). Fish captured from the Hylebos Waterway were held alive in aerated sea water until necropsies could be completed in the shipboard laboratory. Fish from hatcheries and reference estuaries were transported to the laboratory in aerated fresh water and sea water respectively, and held alive until necropsies could be completed.

Fish were weighed (g) and fork length was measured (mm). Bile, liver and stomach contents were collected as described in Varanasi et al. (1993), except that whole livers were collected and later subdivided for each type of analysis. Liver and stomach contents were composited into glass 20-mL vials previously rinsed with methylene chloride. Bile was composited into 4-mL vials containing glass limited-volume inserts. As soon as a composite was completed, it was immediately transferred to a freezer, or maintained on dry ice until it could be transferred to a freezer for storage. Liver samples were stored at –80 _C, and bile and stomach contents were stored at –20 _C until analyses were conducted.

We attempted to collect four composites/species at each site for every sampling period, however, the number of composites collected was dependent on the number of fish available. Each sample was a composite of tissue from 100–150 fish for chum salmon and 30–60 fish for chinook salmon. The number of fish included in a composite was dependent on the amount of tissue needed for analysis, size of the fish, and number of fish available.

Sampling in the Hylebos Waterway was ended when outmigrating salmon were no longer being captured in sufficient numbers to complete a composite for analysis. Due to the low numbers of composites collected at individual reference sites, data from analyses of fish collected at reference sites were combined prior to statistical analyses.

Sample Analysis

Fish liver was analyzed for a number of chlorinated hydrocarbons (CHs) using the methods described by Sloan et al. (1993). CHs most characteristic of the Hylebos Waterway are reported in this paper, and include HCB, HCBD, and sum of PCBs. Analyses were also done for toxic PCB congeners 105 and 118, sum of DDTs, chlordane, lindane, heptachlor, dieldrin and aldrin, but data for these analytes are not included in this paper. Stomach contents were analyzed for high and low molecular weight aromatic hydrocarbons as well the chlorinated hydrocarbons listed above, also using the methods of Sloan et al. (1993). Bile fluorescent aromatic compounds (FACs), including benzo[a]pyrene (BaP), phenanthrene (PHN) and naphthalene (NPH) equivalents, were analyzed by HPLC according to the methods of Krahn et al. (1986). Subsets of liver tissue were also analyzed for CYP1A activity (Collier et al. 1995) and for the presence of DNA adducts (Reichert and French, 1994).

Statistical Methods

Because environmental chemical concentrations and biomarker data are generally log-normally distributed, the data obtained from analyses of FACs in bile, CYP1A and DNA adducts in liver, and organic chemicals in liver and stomach contents were log-transformed prior to statistical analyses (Varanasi et al. 1995, Collier et al. 1986). Since zero values cannot be log-transformed, those values that were below detection limits were statistically analyzed by using 50% of the below detect value. This is a standard method of working with below detection limit values used by our laboratory and others (Bauer et al. 1992). Analysis of variance (ANOVA) was used to determine the statistical significance of differences between the combined data for fish collected during all sampling periods at the Hylebos Waterway and the combined data for reference fish.

Due to the low numbers of composites collected at individual reference sites, data from analyses of

fish collected at reference sites were combined prior to statistical analyses. Scheffe's multiple comparison test showed no significant differences between the reference estuary and hatchery fish.

Results

Juvenile chum and chinook salmon captured in the Hylebos Waterway had significantly higher ($p<0.0001$) concentrations of hexachlorobenzene (HCB), hexachlorobutadiene (HCBD), and the sum of (Σ) polychlorinated biphenyls (PCBs) in their liver compared to juvenile salmon from reference estuaries and hatcheries (Tables 1 and 2).

Table 1. Concentrations of chlorinated hydrocarbons in juvenile chum salmon liver presented as mean concentration ±1 standard error (SE). N = number of samples analyzed. ANOVA was used to test for statistical differences between Hylebos Waterway fish and reference fish. Differences are considered statistically significant at *p* values ≤ 0.05 (in bold). Units of measure are ppb (ng/g) wet weight. * = some or all of the samples in this group had concentrations that were below detection limits; 50% of the below-detection value was used for statistical analyses. Reference sites are Puyallup Tribal hatchery and Skokomish estuary.

Analyte	Tissue	Mean conc. ± SE (N) for Hylebos	Mean conc. ± SE (N) for reference	*p* value
HCB	Liver	5.3 ± 0.4 (12)	0.52± 0.09 (6)	***p* < 0.0001**
HCBD	Liver	2.5 ± 0.2 (12)	*0.08 ± 0.02 (6)	***p* < 0.0001**
SPCBs	Liver	340 ± 20 (12)	40 ± 2.5 (6)	***p* < 0.0001**

Table 2. Concentrations of chlorinated hydrocarbons in juvenile chinook salmon liver. Legend information and description of abbreviations and symbols is the same as for Table 1. Reference sites are Puyallup state hatchery, Nisqually hatchery, and Nisqually estuary.

Analyte	Tissue	Mean conc. ± SE (N) for Hylebos	Mean conc. ± SE (N) for reference	*p* value
HCB	Liver	2.3 ± 0.5 (8)	0.6 ± 0.1 (11)	***p* < 0.0001**
HCBD	Liver	2.3 ± 0.8 (8)	*0.1 ± 0.06 (11)	***p* < 0.0001**
SPCBs	Liver	130 ± 13 (8)	39 ± 5 (11)	***p* < 0.0001**

Many of the chum and chinook juvenile salmon had little or no stomach contents, therefore, the amount of sample available for analyses allowed only one analysis per site for each of two reference sites, and two analyses for salmon from the Hylebos Waterway. Accordingly, only limited statistical analyses could be done with these few data points, and thus, for this study, the chemical analysis of stomach contents should be regarded as a qualitative indicator of contaminant exposure.

Concentrations of the sum of (Σ) high molecular weight aromatic compounds (HACs), HCBD, and ΣPCBs in stomach contents of juvenile chum salmon were significantly higher ($p< 0.05$) in Hylebos Waterway fish compared to reference fish (Table 3).

Table 3. Concentrations of aromatic and chlorinated hydrocarbons in juvenile chum salmon stomach contents. Legend information and description of abbreviations and symbols is the same as for Table 1. Reference sites are Puyallup Tribal hatchery and Skokomish estuary.

Analyte	Tissue	Mean conc. ± SE (N) for Hylebos	Mean conc. ± SE (N) for reference	*p* value
SLACs	Stom. Cont	540 ± 390 (2)	25 ±1 (2)	*p* = 0.1
SHACs	Stom. Cont	1300 ± 740 (2)	4.7 ± 1.1 (2)	***p* = 0.01**
HCB	Stom. Cont	4.4 ± 1.1 (2)	0.7 ± 0.6 (2)	*p* = 0.20
HCBD	Stom. Cont	1.4 ± 0.4 (2)	*0.08 ± 0.03 (2)	***p* = 0.03**
SPCBs	Stom. Cont	140 ± 10 (2)	41 ± 10 (2)	***p* = 0.04**

Mean concentrations of HACs, and HCBD in stomach contents of chinook salmon were significantly

higher ($p<0.05$) (Table 4) in fish captured from the Hylebos Waterway compared to fish from the reference areas (Nisqually River estuary and the Puyallup state hatchery). Although not statistically significant, mean concentrations of low molecular weight aromatic compounds (LACs) and HCB in stomach contents appeared higher in Hylebos fish compared to reference juvenile chinook salmon (Table 4).

Table 4. Concentrations of aromatic and chlorinated hydrocarbons in juvenile chinook salmon stomach contents. Legend information and description of abbreviations and symbols is the same as for Table 1. Reference sites are Puyallup state hatchery and Nisqually estuary.

Analyte	Tissue	Mean conc. ± SE (N) for Hylebos	Mean conc. ± SE (N) for reference	*p* value
SLACs	Stom. Cont	1200 ± 590 (2)	41 ± 32 (2)	***p* = 0.01**
SHACs	Stom. Cont	1800 ± 910 (2)	4 ± 1 (2)	***p* = 0.009**
HCB	Stom. Cont	2.8 ± 1.0 (2)	1.4 ± 1.1 (2)	*p* = 0.43
HCBD	Stom. Cont	1.0 ± 0.5 (2)	0.07 ± 0.01 (2)	***p* = 0.03**
SPCBs	Stom. Cont	92 ± 19 (2)	83 ± 57 (2)	*p* = 0.69

Concentrations of ΣPCBs in stomach contents of juvenile chinook salmon were not significantly different between the reference samples and the Hylebos Waterway samples. This is primarily because of the ΣPCB concentrations observed in stomach contents of chinook sampled from the hatchery. Although there were not enough samples for statistical analyses between the Hylebos Waterway and the Nisqually estuary fish, ΣPCBs in stomach contents of Hylebos Waterway chinook salmon were increased when compared only to chinook salmon from the Nisqually estuary.

Concentrations of metabolites of aromatic hydrocarbons in bile (biliary FACs) of chum salmon (Table 5) measured at benzo(a)pyrene wavelengths ($FACs_{BaP}$; a semi-quantitative estimate of metabolites of HACs), and phenanthrene and naphthalene wavelengths ($FACs_{PHN}$ and $FACs_{NPH}$ respectively; semi-quantitative estimates of metabolites of LACs), were all significantly higher ($p<0.0001$) in Hylebos Waterway fish compared to reference fish.

Table 5. Levels of biochemical indicators of contaminant exposure in juvenile chum salmon. Legend information and description of abbreviations and symbols is the same as for Table 1. Reference sites are Puyallup Tribal hatchery and Skokomish estuary. Units of measure are ng/g bile for bile analyses, pmol/mg/min for CYP1A, and nmol DNA adducts/mol DNA bases for DNA adducts.

Analyte	Tissue	Mean conc. ± SE (N) for Hylebos	Mean conc. ± SE (N) for reference	*p* value
FAC_{BaP}	bile	2200 ± 210 (16)	500 ± 78 (8)	***p* < 0.0001**
FAC_{NPH}	bile	310000 ± 26000 (16)	62000 ± 6900 (8)	***p* < 0.0001**
FAC_{PHN}	bile	78000 ± 7300 (16)	9500 ± 1300 (8)	***p* < 0.0001**
CYP1A	liver	88 ± 11 (12)	29 ± 4 (6)	***p* < 0.0001**
DNA adducts	liver	*8.1 ± 1.3 (12)	*2.7 ± 0.2 (5)	***p* = 0.008**

Concentrations of biliary FACs in juvenile chinook salmon (Table 6) measured at BaP, NPH, and PHN wavelengths were also statistically significant at $p<0.0001$ in Hylebos Waterway fish compared to reference fish.

Cytochrome P4501A (CYP1A), is a xenobiotic metabolizing enzyme inducible by a broad range of PAHs, CHs, and pesticides, and was measured as the activity of the CYP1A-dependent enzyme, aryl hydrocarbon hydroxylase (AHH). These activities were significantly higher ($p<0.0001$) in liver tissue of juvenile chum salmon from the Hylebos Waterway, compared to values for reference fish (Table 5). For chinook salmon, hepatic AHH activities were approximately 30% higher in fish from the Hylebos Waterway, compared to values for reference chinook (Table 6). This difference was statistically significant at $p=0.1$.

Concentrations of DNA adducts in liver, measured by the ^{32}P-postlabeling method, serve as a biological indicator of DNA damage due to exposure to, metabolism of, and covalent binding to DNA bases by PAHs.

These adduct levels were about three times as high in juvenile chum salmon from the Hylebos compared to reference fish, and were statistically significant at p=0.008 (Table 5). However, juvenile chinook salmon showed no significant differences in levels of hepatic DNA adducts between fish captured from the Hylebos Waterway, compared to juvenile chinook captured from the reference sites (Table 6).

Table 6. Levels of biochemical indicators of chemical contaminant exposure in juvenile chinook salmon. Legend information and description of abbreviations and symbols is the same as for Table 1. Reference sites are Puyallup state hatchery, Nisqually hatchery and Nisqually estuary. Units of measure are ng/g bile for bile analyses, pmol/mg/min for CYP1A, and nmol DNA adducts/mol DNA bases for DNA adducts.

Analyte	Tissue	Mean conc. ± SE (N) for Hylebos	Mean conc. ± SE (N) for reference	*p* value
FAC_{BaP}	bile	2800 ± 490 (10)	1000 ± 100 (11)	***p* < 0.0001**
FAC_{NPH}	bile	520000 ± 86000 (10)	120000 ± 9100 (11)	***p* < 0.0001**
FAC_{PHN}	bile	110000 ± 19000 (10)	19000 ± 1900 (11)	***p* < 0.0001**
DNA add.	liver	*6.0 ± 1.1 (8)	*6.4 ± 0.7 (11)	*p* = 0.68
CYP1A	liver	97 ± 15 (8)	64 ± 13 (11)	*p* = 0.10

Discussion

Juvenile chum and chinook salmon from the Hylebos Waterway show an increased exposure to chemical contaminants, compared to fish from hatcheries or reference estuaries. The liver, stomach contents, and bile of both chum and chinook salmon from the Hylebos Waterway showed increased concentrations of aromatic compounds and their metabolites, and to HCB, HCBD, and ΣPCBs. Other chlorinated chemicals including toxic PCB congeners 105 and 118, ΣDDTs, hexachlor, lindane, dieldrin, aldrin, and chlordane, were also generally elevated in the liver of Hylebos Waterway juvenile salmon (Collier et al. 1998). There was also evidence of low to moderate contamination with chlorinated compounds in the feed used at the hatcheries, as shown by analyses of stomach contents (only one analysis per hatchery was conducted). However, it is apparent that any such contamination is not a major factor in the increased body burdens measured in fish from the Hylebos Waterway, because levels of PCBs and chlorinated pesticides in the liver were clearly elevated in fish captured from the Hylebos Waterway, compared to fish from either the hatcheries or other contaminated estuaries. The presence of high levels of HCBD in liver tissue and stomach contents provides strong evidence that exposure of these animals originates from the Hylebos Waterway, rather than from other waterways in Commencement Bay, because this compound is found in high levels in the sediments of the lower Hylebos Waterway (Collier et al. 1998; EVS, 1996; Malins et al. 1982), with dramatically lower levels found elsewhere in the Commencement Bay ecosystem (Malins et al. 1982; Krahn, pers. comm.). In fact, liver concentrations of HCBD in juvenile chum and chinook exceed those found in any previous studies of juvenile salmonids (Varanasi et al. 1993).

Associated with these increased concentrations of chemicals, there are indications of early biological alterations and damage, as shown by the increases in hepatic CYP1A-associated enzyme activity in both species and increased levels of DNA damage in chum salmon. Increases in both of these measures are well established as being linked to contaminant exposure (Collier and Varanasi 1991; Varanasi et al. 1992; Stein et al. 1992). However, the measurement of DNA damage is less sensitive than induction of CYP1A for determining comparatively short-term exposure to moderate levels of contaminants (Collier et al. 1988), which is likely the case for juvenile salmon inhabiting the Hylebos Waterway during their acclimation to marine conditions.

It is notable that chum salmon from the Hylebos Waterway generally showed higher indices of exposure than did chinook salmon. Whether this is due to increased exposure, decreased elimination, or other species-specific factors cannot be determined from the current data. Nonetheless, the apparent higher contaminant concentrations in chum salmon raises the possibility that this species may be more susceptible to contaminant-induced biological injury than chinook salmon.

Concentrations of contaminants in juvenile chinook and chum salmon from the Hylebos Waterway are comparable to levels previously observed in juvenile chinook salmon from other industrial waterways (Figure 1). These contaminant concentrations have been shown to be associated with biological injury in juvenile chinook salmon. Contaminant concentrations similar to those measured in liver, stomach contents, and bile of juvenile salmon from the Hylebos Waterway are associated with impaired growth, suppression of immune function, and increased mortality following pathogen exposure in chinook salmon collected from another contaminated estuary in Puget Sound, the Duwamish Waterway (Varanasi et al. 1993; Arkoosh et al. 1991; Arkoosh et al. in press) For most measures in the current study, the concentrations of contaminants in Hylebos Waterway juvenile salmon are similar to, or in the case of HCBD, substantially higher than, concentrations measured in Duwamish Waterway fish (Figure 1).

As salmon complete smoltification and move from freshwater habitats to estuarine and marine habitats, they must adapt to a range of different pathogens and prey organisms, and are also subject to predation from different predators. Thus, impaired abilities to withstand pathogenic challenges and altered growth patterns should generally be considered to be deleterious with respect to early ocean survival of juvenile salmon utilizing contaminated habitats.

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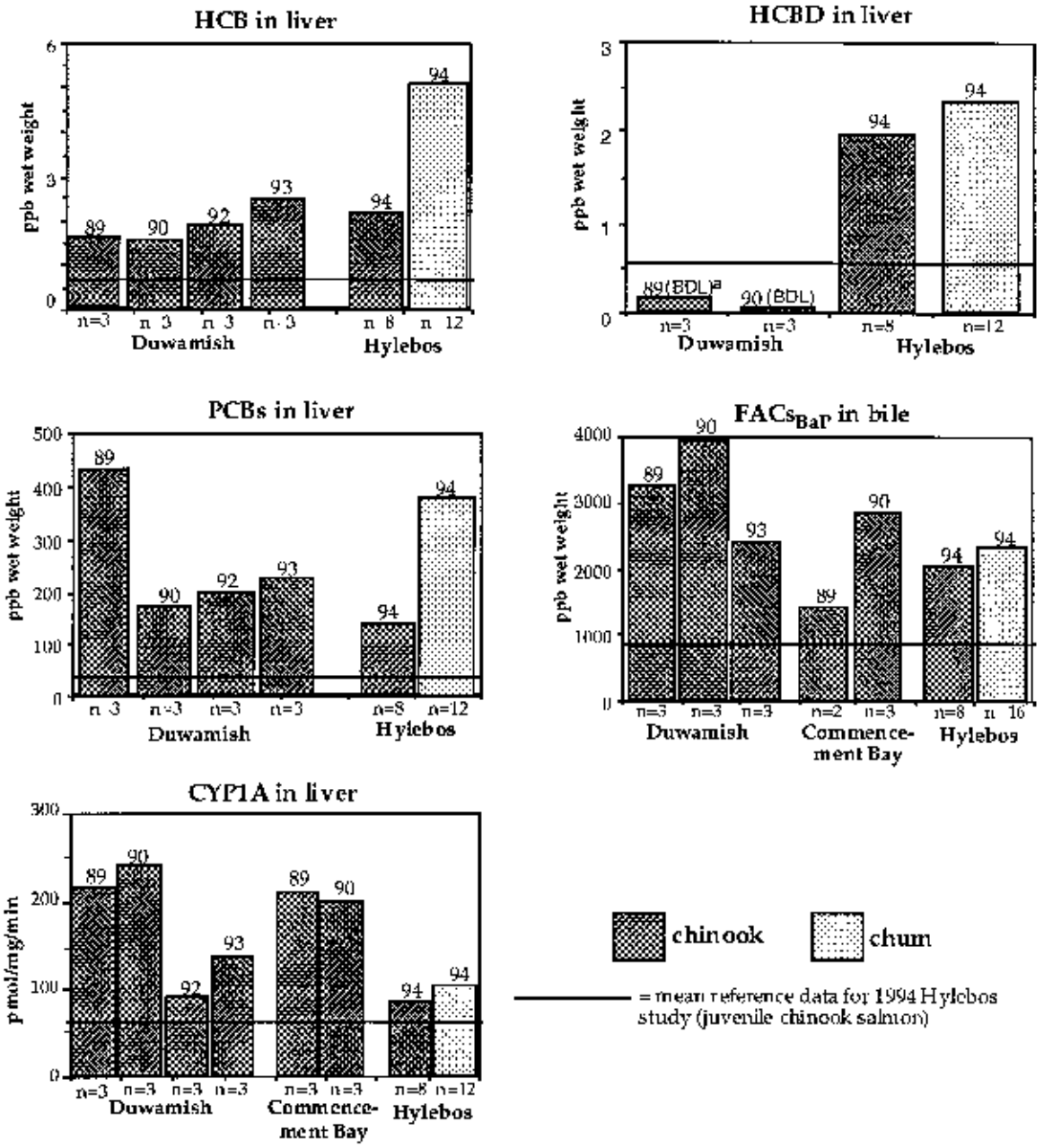

Figure 1. Comparisons of 1994 Hylebos Waterway juvenile salmon data with historical data from Puget Sound, WA. (Year of study is listed above each bar; the number [n] of composites analyzed at each site is listed below each bar.)

[a]BDL=all of the samples had concentrations that were below detection limits, therefore values were treated as if the concentration was 50% of the detection limit. (Sources: Duwamish and Commencement Bay 1989 and 1990 data from Stein et al. [1995] and Varanasi et al. [1993]. Duwamish 1992 and 1993 data from Casillas [unpublished data].)

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